

XTO ENERGY INC

52260 Wik Road
Kenai, Alaska 99611

NPDES - Discharge Monitoring Report

| <u>Facility</u> | <u>Permit ID</u> | <u>Month</u> |
|-----------------|------------------|--------------|
| Platform A | AKG-31-5012 | Jun-12 |

Attachments

- ◆ 1st Period 2012 WET Test Reports & Annual Re-Screen

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-129Title: Inland silverside, *Menidia beryllina*, chronic toxicity test using static renewal exposure to XTO Energy Platform A water flood sample.Protocol No.: NAS-XXX-MB2, September 15, 1990, Revision 2 (2-8-08). Based on U.S. EPA, 2002. Method 1006.0, Inland Silverside, *Menidia beryllina*, larval survival and growth test, pp. 155-213. In: Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA-821-R-02-014.

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd., Kenai, AK 99611.Sponsor's Study Monitor: Mr. Ryan TunsethTesting Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.Test Location: Newport Laboratory.Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Mgr./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; G.J. Irissarri, B.S., Aq. Toxicol.; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Sr. Tech.Study Schedule:

Test Beginning: 5-1-12, 1120 hrs.

Test Ending: 5-8-12, 1025 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy Platform A water flood sample. Details follow:

| | | | |
|-------------------------|---------|--------|--------|
| NAS Sample No. | 4068G | 4073G | 4078G |
| Collection Date | 4-30-12 | 5-2-12 | 5-4-12 |
| Receipt Date | 5-1-12 | 5-3-12 | 5-5-12 |
| Temperature (°C) | 2.3 | 2.2 | 1.7 |
| pH | 8.1 | 8.0 | 7.9 |
| Dissolved oxygen (mg/L) | 10.1 | 10.7 | 3.8 |
| Salinity (‰) | 28.0 | 29.0 | 29.0 |

Treatments: Samples were briefly temperature equilibrated prior to use.Storage: Stored at 4°C in the dark until used.

DILUTION WATER

Source: Yaquina Bay, Oregon seawaterDate of Collection: 4-29-12Water Quality: Salinity, 30.0 ‰; pH 8.2Pretreatment: Filtered to ≤0.45 µm, salinity adjusted with Milli-Q water and aerated.

TEST ORGANISMS

Species: *Menidia beryllina*, inland silversideAge: 11 days post hatchSource: Aquatic Indicators Inc., St. Augustine, Florida.Acclimation: Fish were received at the laboratory four days before testing. During acclimation, silverside larvae were fed *Artemia nauplii* daily and 50% of the holding water was changed daily. The mean of holding conditions,

including receiving water, prior to testing averaged: temperature, $23.5 \pm 0.8^{\circ}\text{C}$; pH, 7.6 ± 0.3 ; salinity, 25.8 ± 2.8 ‰; and dissolved oxygen, 7.5 ± 4.2 mg/L.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 1,000 ml glass beakers containing 500 ml of test solution

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01 and 0 % (control).

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.037 g/L

Aeration: None.

Feeding: Approximately 0.1 g newly hatched *Artemia* nauplii per beaker twice daily, except on day 7.

Water Volume Changes: once daily

Acceptance Criterion: Results are valid if mean control survival is at least 80%, and the average dry weight of control larvae at test termination is at least 0.50 mg (based on number of surviving fish, where fish are 7 days old at test initiation).

Effects Criteria The effect criteria used were: 1) mortality, and 2) growth inhibition. Mortality was defined as lack of visible movement during a 30 second observation period. Growth inhibition was measured as the difference in weight of fish between a treatment level and the control.

Water Quality and Other Test Conditions: Temperature, $24.9 \pm 0.2^{\circ}\text{C}$; pH, 8.0 ± 0.1 ; salinity, 30.1 ± 0.2 ‰; dissolved oxygen, 6.0 ± 0.6 mg/L and photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

Percent survival and the average weight per larva were calculated for each treatment replicate from the raw data and the means were obtained for each treatment level. Average weights were calculated based on the initial number of fish. The LC50 (survival) was calculated, where data permitted, either by the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber method. The IC25 (growth) was calculated using the Linear Interpolation Method with bootstrapping. NOEC and LOEC values for survival and growth were computed using ANOVA and an appropriate post hoc test (Dunnett's test, T-Test with Bonferroni's adjustment, Steels Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment). The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine square root (angular) transformation was performed on the survival data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v.1.7.0revW, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/LOEC, 100/LC50, or 100/IC25.

PROTOCOL DEVIATIONS

None.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3034

Reference Toxicant and Source: Copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Argent Lot No. 0195, 1.0 mg/mL stock prepared 6-28-10.

Test Date: 5-1-12

Dilution Water Used: Yaquina Bay, Oregon, seawater; salinity 12.0 ‰ and pH 8.0

Results: LC50, 22.7 µg/L; NOEC, 15 µg/L; IC25, 18.0 µg/L. These results are within the laboratory's control chart warning limits (LC50, 8.36 – 38.0 µg Cu/L; IC25, 7.58 – 28.4 µg Cu/L).

TEST RESULTS

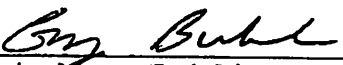

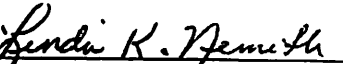
A detailed tabulation of the test results is given in Table 1. The biological effects, given as the NOEC and LOEC for survival and growth, and the LC50/IC25 for survival/growth, are shown below.

| | | |
|------------------------|----------------------|-------------------------|
| <u>Survival</u> | | |
| NOEC (%) | 0.16 | (TU _c = 625) |
| LOEC (%) | >0.16 | (TU _c <625) |
| LC50 (%) | >0.16 | (TU _c <625) |
| (95% C.I.) | — | — |
| Method of Calculation | Data Inspection | |
| <u>Growth</u> | | |
| NOEC (%) | 0.16 | (TU _c = 625) |
| LOEC (%) | >0.16 | (TU _c <625) |
| IC25 (%) | >0.16 | (TU _c <625) |
| (95% C.I.) | — | — |
| Method of Calculation | Linear Interpolation | |

DISCUSSION/CONCLUSIONS

Comments: Seven-day control survival (92.5%) and mean control weight (1.98 mg based on number of surviving fish) met the test acceptability criteria of $\geq 80\%$ and ≥ 0.50 mg, respectively. The reference toxicant test results were within control chart limits. Therefore, this toxicity test is considered a valid test.

STUDY APPROVAL

| | |
|---|--|
|  |  |
| Project Manager/Study Director | Quality Assurance Unit |
|  | |
| Assistant Laboratory Director | |

6/20/12 6-21-12

Date Date

6/20/12

Date

Table 1. Survival and growth of inland silverside, *Menidia beryllina*, larvae exposed for seven days to XTO Energy Platform A water flood sample.

| Effluent Concentration (%) | Replicate | Number of larvae | | | % Survival | Mean % Survival * | Average wt./larva (mg) | Mean wt. (mg) * |
|----------------------------------|-----------|------------------|------|-----------|---------------|-------------------------|------------------------------|-----------------------|
| | | Exposed | Dead | Surviving | | | | |
| 0.16 | 1 | 10 | 2 | 8 | 80.0 | | 2.312 | |
| | 2 | 10 | 2 | 8 | 80.0 | | 2.298 | |
| | 3 | 10 | 2 | 8 | 80.0 | | 2.598 | |
| | 4 | 10 | 1 | 9 | 90.0 | 82.5 | 2.505 | 2.428 |
| 0.08 | 1 | 10 | 1 | 9 | 90.0 | | 2.614 | |
| | 2 | 10 | 0 | 10 | 100 | | 2.566 | |
| | 3 | 10 | 1 | 9 | 90.0 | | 2.443 | |
| | 4 | 10 | 0 | 10 | 100 | 95.0 | 2.596 | 2.555 |
| 0.04 | 1 | 10 | 0 | 10 | 100 | | 1.912 | |
| | 2 | 10 | 0 | 10 | 100 | | 2.380 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.830 | |
| | 4 | 10 | 0 | 10 | 100 | 100 | 2.724 | 2.462 |
| 0.02 | 1 | 10 | 1 | 9 | 90.0 | | 2.016 | |
| | 2 | 10 | 1 | 9 | 90.0 | | 2.051 | |
| | 3 | 10 | 1 | 9 | 90.0 | | 1.933 | |
| | 4 | 10 | 0 | 10 | 100 | 92.5 | 1.980 | 1.995 |
| 0.01 | 1 | 10 | 1 | 9 | 90.0 | | 2.025 | |
| | 2 | 10 | 2 | 8 | 80.0 | | 1.824 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.437 | |
| | 4 | 10 | 0 | 10 | 100 | 92.5 | 2.058 | 2.086 |
| 0 control | 1 | 10 | 0 | 10 | 100 | | 1.916 | |
| | 2 | 10 | 2 | 8 | 80.0 | | 1.870 | |
| | 3 | 10 | 1 | 9 | 90.0 | | 1.841 | |
| | 4 | 10 | 0 | 10 | 100 | 92.5 | 1.702 | 1.832 |

* An asterisk next to a treatment mean indicates that it is significantly ($P < 0.05$) less than the control mean.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-139

Title: Echinoderm sperm-fertilization test using static exposure to XTO Energy Platform A water flood sample.

Protocol No.: NAS-XXX-SP/DE2, August 10, 1990 (Revision 3, 10-24-02). Based on: Method 1008.0, Sea Urchin, *Strongylocentrotus purpuratus*, and Sand Dollar, *Dendraster excentricus*, fertilization test, pp. 389-465, In: Short term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms, EPA/600/R-95/136.

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd., Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Mgr./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 5-3-12, 1500 hrs.

Test Ending: 5-3-12, 1540 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy Platform A water flood sample. Details follow:

| | |
|-------------------------|--------|
| NAS Sample No. | 4073G |
| Collection Date | 5-2-12 |
| Receipt Date | 5-3-12 |
| Temperature (°C) | 2.2 |
| pH | 8.0 |
| Dissolved oxygen (mg/L) | 10.7 |
| Salinity (‰) | 29.0 |

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, OR

Date of Collection: 5-2-12

Water Quality: Salinity: 34.0 ‰, pH: 8.0

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, aerated, salinity adjusted with 100 ppt brine prepared on 3-19-12.

BRINE USED FOR SALINITY CONTROL

None used.

TEST ORGANISMS

Species: Sea urchin (*Strongylocentrotus purpuratus*).

Age: Sperm were used immediately after seawater activation.

Source: Marinus Scientific, Newport Beach, CA.

Acclimation: Adults were received on 2-1-12 and held in flowing seawater until used for testing. Holding conditions prior to testing averaged: temperature, $11.9 \pm 0.9^\circ\text{C}$; pH, 8.0 ± 0.1 ; salinity, 26.2 ± 2.7 ppt; and dissolved oxygen, 8.2 ± 0.7 mg/L.

Source of Gametes: 1 female, 2 males

TEST PROCEDURES AND CONDITIONS

Test Chambers: 16 mm x 100 mm unwashed new borosilicate disposable glass test tubes containing 5 ml of test solution.

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01 and 0% (control).

Brine Control: None used

Replicates/Treatment: 4

Eggs per Test Container: 1000

Sperm:Egg Ratio: 100:1

Sperm Exposure Time: 20 minutes

Time for Fertilization: 20 minutes

Volume of Subsamples Taken for Counting: 1 ml

Water Volume Changes: None (non-renewal static test).

Aeration: None

Feeding: None

Effects Criteria: The effect criterion was absence of fertilization as indicated by lack of a fertilization membrane in the preserved eggs.

Water Quality and Other Test Conditions: Temperature, 11.8°C ; pH, 8.0 ± 0.0 ; salinity, 33.9 ± 0.2 ‰; and dissolved oxygen, 8.5 ± 0.1 mg/L. Photoperiod: NA

DATA ANALYSIS METHODS

The proportion of fertilized eggs was calculated for each treatment replicate from the raw data and the means were obtained for each treatment level. The latter were then corrected for control response using Abbott's formula. The EC50 was calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber methods. An IC25 was calculated by linear interpolation with bootstrapping. NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v.1.7.0revW, Tidepool Scientific Software. Toxic units (TU_c) were computed as $100/\text{NOEC}$, $100/\text{EC50}$, or $100/\text{IC25}$.

PROTOCOL DEVIATIONS

None.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using sodium azide to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3025

Reference Toxicant and Source: Sodium azide (Sigma Lot No. 68F-0834), 1.0 mg/mL stock prepared on 5-3-12.

Test Date: 5-3-12

Dilution Water Used: Yaquina Bay, OR, Salinity 34.0 ‰, pH 8.0.

Results: EC50, 191 mg/L; NOEC, 47 mg/L; and IC25, 148

mg/L. The EC50 result is slightly above the laboratory's control chart warning limits (EC50, 83.2 – 189 mg/L), but is within the control chart upper action limit (216 mg/L). Control limits of ± 2 SD will be exceeded 5% of the time by chance alone, and there was no evidence that these organisms were unusual in any way.

TEST RESULTS

A detailed tabulation of the test results is given in Table 1. The biological effects, given as the NOEC, LOEC, and EC50 and IC25 for inhibition of fertilization are shown below.

| | |
|-----------------------|--------------------------|
| NOEC (%) | 0.16 ($TU_c = 625$) |
| LOEC (%) | >0.16 ($TU_c < 625$) |
| EC50 (%) | >0.16 ($TU_c < 625$) |
| (95% C.I.) | -- |
| Method of Calculation | By Data Inspection |
| IC25 (%) | >0.16 ($TU_c < 625$) |
| (95% C.I.) | -- |
| Method of Calculation | Linear Interpolation |

DISCUSSION/CONCLUSIONS

The NOEC in this study was 0.16 % effluent, and the EC50 and IC25 for fertilization were both >0.16 %.

The reference toxicant test results exceeded the control chart limits for the EC50. The EC50 was 191 mg/L, which is slightly above the upper warning limit of 189 mg/L, but within the upper action limit of 216 mg/L. No other problems could be found with the test organisms or the testing procedure.

Table 2 shows the results of the egg-effluent control and egg-control tests. No fertilization response was observed in the egg only, no-sperm control or in the egg-effluent, no-sperm control.

STUDY APPROVAL

Gary Buhle
Project Manager/Study Director

6/19/12
Date

Julie R. Fiske
Quality Assurance Unit

6-19-12
Date

Linda K. Demuth
Assistant Laboratory Director

6/15/12
Date

Table 1. Fertilization response of Sea urchin, *Strongylocentrotus purpuratus*, sperm exposed to XTO Energy Platform A water flood sample.

| Effluent Conc. (%) | Replicate | Eggs Counted | | Proportion Fertilized | |
|--------------------|-----------|--------------|--------------|-----------------------|-------|
| | | Fertilized | Unfertilized | Mean** | |
| 0.16 | 1 | 98 | 2 | 0.980 | 0.988 |
| | 2 | 97 | 3 | 0.970 | |
| | 3 | 100 | 0 | 1.000 | |
| | 4 | 100 | 0 | 1.000 | |
| 0.08 | 1 | 97 | 3 | 0.970 | 0.983 |
| | 2 | 98 | 2 | 0.980 | |
| | 3 | 100 | 0 | 1.000 | |
| | 4 | 98 | 2 | 0.980 | |
| 0.04 | 1 | 98 | 2 | 0.980 | 0.990 |
| | 2 | 99 | 1 | 0.990 | |
| | 3 | 99 | 1 | 0.990 | |
| | 4 | 100 | 0 | 1.000 | |
| 0.02 | 1 | 99 | 1 | 0.990 | 0.985 |
| | 2 | 98 | 2 | 0.980 | |
| | 3 | 99 | 1 | 0.990 | |
| | 4 | 98 | 2 | 0.980 | |
| 0.01 | 1 | 99 | 1 | 0.990 | 0.978 |
| | 2 | 96 | 4 | 0.960 | |
| | 3 | 97 | 3 | 0.970 | |
| | 4 | 99 | 1 | 0.990 | |
| Control | 1 | 85 | 15 | 0.850 | 0.948 |
| | 2 | 98 | 2 | 0.980 | |
| | 3 | 97 | 3 | 0.970 | |
| | 4 | 99 | 1 | 0.990 | |

** Treatment mean significantly ($P < 0.05$) different from the control mean

Table 2. Response of egg-effluent controls (no sperm) and egg-controls (no sperm, dilution water only).

| Description | Replicate | Eggs Counted | | Proportion Fertilized | |
|----------------------|-----------|--------------|--------------|-----------------------|-------|
| | | Fertilized | Unfertilized | Mean | |
| Egg-effluent control | 1 | 0 | 100 | 0.000 | 0.000 |
| | 2 | 0 | 100 | 0.000 | |
| | 3 | 0 | 100 | 0.000 | |
| | 4 | 0 | 100 | 0.000 | |
| Egg control | 1 | 0 | 100 | 0.000 | 0.000 |
| | 2 | 0 | 100 | 0.000 | |
| | 3 | 0 | 100 | 0.000 | |
| | 4 | 0 | 100 | 0.000 | |

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-144

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform A – water flood.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Aq. Toxicologist; M.S. Redmond, M.S., Aq. Toxicol.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 5-5-12, 1320 hrs.

Test Ending: 5-7-12, 1430 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy – Platform A – water flood. Details are as follows:

| | |
|-------------------------|--------|
| NAS Sample No. | 4078G |
| Collection Date | 5-4-12 |
| Receipt Date | 5-5-12 |
| Temperature (°C) | 1.7 |
| pH | 7.9 |
| Dissolved oxygen (mg/L) | 3.8 |
| Salinity (‰) | 29.0 |

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 5-4-12

Water Quality: Salinity, 30.5 ‰; pH, 8.1

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, aerated, and salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 1.7 hours post-fertilization.

Source: Carlsbad Aquafarm, Carlsbad, CA.

Conditioning: Adult mussels were received on 5-5-12 and used directly from shipping container.

Source of Gametes: 4 females and 2 males.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).

Brine Control: None used

Replicates/Treatment: 4

Initial Concentration of Test Organisms: 20.3/ml.

Volume of Subsamples Taken for Counting: NA

Water Volume Changes per 24 hr: None (non-renewal static test).

Aeration: None

Feeding: None

Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.

Water Quality and Other Test Conditions: Temperature, $15.8 \pm 0.1^\circ\text{C}$; pH, 8.1 ± 0.1 ; salinity, 30.0 ± 0.3 ‰; and dissolved oxygen, 8.1 ± 0.0 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0revW, Tidepool Scientific Software. Toxic units (TU_c) were computed as $100/\text{NOEC}$, $100/\text{EC50}$, or $100/\text{IC25}$.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3039

Reference Toxicant and Source: Copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Argent Lot No. 0195. Concentrated stock prepared 6-28-10.

Test Date: 5-5-12

Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.5 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.41 µg/L. The EC50 results are within the laboratory's control chart warning limits (9.17 – 12.4 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

| | Combined Proportion Normal | Survival |
|-----------------------------|-------------------------------|----------------------|
| NOEC (%) | 0.16 ($TU_c=625$) | 0.16 ($TU_c=625$) |
| LOEC (%) | >0.16 ($TU_c<625$) | >0.16 ($TU_c<625$) |
| EC50/LC50 (%) (95% C.I.) | >0.16 ($TU_c<625$) | >0.16 ($TU_c<625$) |
| Method of Calculation | By Data Inspection | By Data Inspection |
| IC25 (%) (95% C.I.) | >0.16 ($TU_c<625$) | --- |
| Method of Calculation | Linear Interpolation | --- |

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Guy Buhl 6/19/12
Project Manager Date

Maab Almasri 6-19-12
Study Director Date

Linda K. Hemmick 6/13/12
Assistant Laboratory Director Date

Julie R. Fier 6-19-12
Quality Assurance Unit Date

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – Platform A – water flood.

| Test Material | | | | | Combined Proportion Normal* | | Proportion Survived* | |
|-------------------|-------|-------|------|-------|-----------------------------|-------|----------------------|-------|
| Concentration (%) | Repl. | Norm. | Abn. | Total | Mean | | Mean | |
| 0.16 | 1 | 178 | 7 | 185 | 0.877 | | 0.911 | |
| | 2 | 203 | 13 | 216 | 1.000 | | 1.000 | |
| | 3 | 232 | 1 | 233 | 0.996 | | 1.000 | |
| | 4 | 231 | 6 | 237 | 0.975 | 0.962 | 1.000 | 0.978 |
| 0.08 | 1 | 193 | 6 | 199 | 0.951 | | 0.980 | |
| | 2 | 178 | 13 | 191 | 0.877 | | 0.941 | |
| | 3 | 185 | 5 | 190 | 0.911 | | 0.936 | |
| | 4 | 213 | 7 | 220 | 0.968 | 0.927 | 1.000 | 0.964 |
| 0.04 | 1 | 195 | 3 | 198 | 0.961 | | 0.975 | |
| | 2 | 205 | 5 | 210 | 0.976 | | 1.000 | |
| | 3 | 223 | 7 | 230 | 0.970 | | 1.000 | |
| | 4 | 179 | 8 | 187 | 0.882 | 0.947 | 0.921 | 0.974 |
| 0.02 | 1 | 203 | 2 | 205 | 1.000 | | 1.000 | |
| | 2 | 185 | 11 | 196 | 0.911 | | 0.966 | |
| | 3 | 200 | 6 | 206 | 0.985 | | 1.000 | |
| | 4 | 178 | 3 | 181 | 0.877 | 0.943 | 0.892 | 0.964 |
| 0.01 | 1 | 170 | 12 | 182 | 0.837 | | 0.897 | |
| | 2 | 193 | 11 | 204 | 0.951 | | 1.000 | |
| | 3 | 220 | 9 | 229 | 0.961 | | 1.000 | |
| | 4 | 176 | 5 | 181 | 0.867 | 0.904 | 0.892 | 0.947 |
| Normal Control | 1 | 210 | 8 | 218 | 0.963 | | 1.000 | |
| | 2 | 173 | 3 | 176 | 0.852 | | 0.867 | |
| | 3 | 197 | 3 | 200 | 0.970 | | 0.985 | |
| | 4 | 171 | 4 | 175 | 0.842 | 0.907 | 0.862 | 0.929 |

* Based on an average initial count of 203 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal > average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different ($P \leq 0.05$) from the control.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-130

Title: Inland silverside, *Menidia beryllina*, chronic toxicity test using static renewal exposure to XTO Energy Platform A non-contact cooling water sample.

Protocol No.: NAS-XXX-MB2, September 15, 1990, Revision 2 (2-8-08). Based on U.S. EPA, 2002. Method 1006.0, Inland Silverside, *Menidia beryllina*, larval survival and growth test, pp. 155-213. In: Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA-821-R-02-014.

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd., Kenai, AK 99611.

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport Laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Mgr./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; G.J. Irissarri, B.S., Aq. Toxicol.; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 5-1-12, 1210 hrs.

Test Ending: 5-8-12, 1130 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy Platform A non-contact cooling water sample. Details follow:

| NAS Sample No. | 4069G | 4074G | 4079G |
|-------------------------|---------|--------|--------|
| Collection Date | 4-30-12 | 5-2-12 | 5-4-12 |
| Receipt Date | 5-1-12 | 5-3-12 | 5-5-12 |
| Temperature (°C) | 2.1 | 2.0 | 1.2 |
| pH | 8.1 | 8.0 | 8.0 |
| Dissolved oxygen (mg/L) | 9.5 | 10.4 | 9.8 |
| Salinity (‰) | 29.5 | 29.0 | 29.0 |

Treatments: Samples were briefly temperature equilibrated prior to use.

Storage: Stored at 4°C in the dark until used.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater

Date of Collection: 4-29-12

Water Quality: Salinity, 30.0 ‰; pH 8.2

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, salinity adjusted with Milli-Q water and aerated.

TEST ORGANISMS

Species: *Menidia beryllina*, inland silverside

Age: 11 days post hatch

Source: Aquatic Indicators Inc., St. Augustine, Florida.

Acclimation: Fish were received at the laboratory four days before testing. During acclimation, silverside larvae were fed *Ariemina nauplii* daily and 50% of the holding water was changed daily. The mean of holding conditions,

including receiving water, prior to testing averaged: temperature, $23.5 \pm 0.8^{\circ}\text{C}$; pH, 7.6 ± 0.3 ; salinity, 25.8 ± 2.8 ‰; and dissolved oxygen, 7.5 ± 4.2 mg/L.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 1,000 ml glass beakers containing 500 ml of test solution

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01 and 0 % (control).

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.054 g/L

Aeration: None.

Feeding: Approximately 0.1 g newly hatched *Artemia* nauplii per beaker twice daily, except on day 7.

Water Volume Changes: once daily

Acceptance Criterion: Results are valid if mean control survival is at least 80%, and the average dry weight of control larvae at test termination is at least 0.50 mg (based on number of surviving fish, where fish are 7 days old at test initiation).

Effects Criteria: The effect criteria used were: 1) mortality, and 2) growth inhibition. Mortality was defined as lack of visible movement during a 30 second observation period. Growth inhibition was measured as the difference in weight of fish between a treatment level and the control.

Water Quality and Other Test Conditions: Temperature, $24.9 \pm 0.2^{\circ}\text{C}$; pH, 7.9 ± 0.1 ; salinity, 30.0 ± 0.1 ‰; dissolved oxygen, 6.0 ± 0.6 mg/L and photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

Percent survival and the average weight per larva were calculated for each treatment replicate from the raw data and the means were obtained for each treatment level. Average weights were calculated based on the initial number of fish. The LC50 (survival) was calculated, where data permitted, either by the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber method. The IC25 (growth) was calculated using the Linear Interpolation Method with bootstrapping. NOEC and LOEC values for survival and growth were computed using ANOVA and an appropriate *post hoc* test (Dunnnett's test, T-Test with Bonferroni's adjustment, Steels Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment). The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine square root (angular) transformation was performed on the survival data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v.1.7.0revW, Tidepool Scientific Software. Toxic units (TU_x) were computed as 100/NOEC, 100/LOEC, 100/LC50, or 100/IC25.

PROTOCOL DEVIATIONS

None.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3034

Reference Toxicant and Source: Copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Argent Lot No. 0195, 1.0 mg/mL stock prepared 6-28-10.

Test Date: 5-1-12

Dilution Water Used: Yaquina Bay, Oregon, seawater; salinity 12.0 ‰ and pH 8.0

Results: LC50, 22.7 µg/L; NOEC, 15 µg/L; IC25, 18.0 µg/L. These results are within the laboratory's control chart warning limits (LC50, 8.36 – 38.0 µg Cu/L; IC25, 7.58 – 28.4 µg Cu/L).

TEST RESULTS

A detailed tabulation of the test results is given in Table 1. The biological effects, given as the NOEC and LOEC for survival and growth, and the LC50/IC25 for survival/growth, are shown below.

Survival

| | | |
|-----------------------|-----------------|-------------------------|
| NOEC (%) | 0.16 | (TU _c = 625) |
| LOEC (%) | >0.16 | (TU _c < 625) |
| LC50 (%) | >0.16 | (TU _c < 625) |
| (95% C.I.) | — | — |
| Method of Calculation | Data Inspection | |

Growth

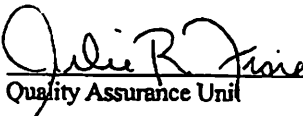
| | | |
|-----------------------|----------------------|-------------------------|
| NOEC (%) | 0.16 | (TU _c = 625) |
| LOEC (%) | >0.16 | (TU _c < 625) |
| IC25 (%) | >0.16 | (TU _c < 625) |
| (95% C.I.) | — | — |
| Method of Calculation | Linear Interpolation | |

DISCUSSION/CONCLUSIONS

Comments: Seven-day control survival (95.0%) and mean control weight (2.84 mg based on number of surviving fish) met the test acceptability criteria of $\geq 80\%$ and ≥ 0.50 mg, respectively. The reference toxicant test results were within control chart limits. Therefore, this toxicity test is considered a valid test.

STUDY APPROVAL


 Project Manager/Study Director 6/21/12
 Date


 Quality Assurance Unit 6-21-12
 Date


 Assistant Laboratory Director 6/20/12
 Date

Table 1. Survival and growth of inland silverside, *Menidia beryllina*, larvae exposed for seven days to XTO Energy Platform A non-contact cooling water sample.

| Effluent Concentration (%) | Replicate | Number of larvae | | | % Survival | Mean % Survival * | Average wt./larva (mg) | Mean wt. (mg) * |
|----------------------------------|-----------|------------------|------|-----------|---------------|-------------------------|------------------------------|-----------------------|
| | | Exposed | Dead | Surviving | | | | |
| 0.16 | 1 | 10 | 1 | 9 | 90.0 | | 2.428 | |
| | 2 | 10 | 1 | 9 | 90.0 | | 2.442 | |
| | 3 | 10 | 2 | 8 | 80.0 | | 2.193 | |
| | 4 | 10 | 0 | 10 | 100 | 90.0 | 2.827 | 2.473 |
| 0.08 | 1 | 10 | 0 | 10 | 100 | | 2.528 | |
| | 2 | 10 | 2 | 8 | 80.0 | | 2.124 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.568 | |
| | 4 | 10 | 0 | 10 | 100 | 95.0 | 2.639 | 2.465 |
| 0.04 | 1 | 10 | 1 | 9 | 90.0 | | 2.235 | |
| | 2 | 10 | 0 | 10 | 100 | | 2.639 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.829 | |
| | 4 | 10 | 1 | 9 | 90.0 | 95.0 | 2.310 | 2.503 |
| 0.02 | 1 | 10 | 1 | 9 | 90.0 | | 2.429 | |
| | 2 | 10 | 1 | 9 | 90.0 | | 1.972 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.518 | |
| | 4 | 10 | 0 | 10 | 100 | 95.0 | 2.805 | 2.431 |
| 0.01 | 1 | 10 | 1 | 9 | 90.0 | | 2.339 | |
| | 2 | 10 | 1 | 9 | 90.0 | | 2.524 | |
| | 3 | 10 | 0 | 10 | 100 | | 2.728 | |
| | 4 | 10 | 0 | 10 | 100 | 95.0 | 3.103 | 2.674 |
| 0 control | 1 | 10 | 0 | 10 | 100 | | 2.830 | |
| | 2 | 10 | 1 | 9 | 90.0 | | 2.348 | |
| | 3 | 10 | 1 | 9 | 90.0 | | 2.755 | |
| | 4 | 10 | 0 | 10 | 100 | 95.0 | 2.873 | 2.702 |

* An asterisk next to a treatment mean indicates that it is significantly ($P < 0.05$) less than the control mean.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-140

Title: Echinoderm sperm-fertilization test using static exposure to XTO Energy Platform A non-contact cooling water sample.

Protocol No.: NAS-XXX-SP/DE2, August 10, 1990 (Revision 3, 10-24-02). Based on: Method 1008.0, Sea Urchin, *Strongylocentrotus purpuratus*, and Sand Dollar, *Dendraster excentricus*, fertilization test, pp. 389-465, In: Short term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms, EPA/600/R-95/136.

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd., Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Mgr./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 5-3-12, 1500 hrs.

Test Ending: 5-3-12, 1540 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy Platform A non-contact cooling water sample. Details follow:

| | |
|-------------------------|--------|
| NAS Sample No. | 4074G |
| Collection Date | 5-2-12 |
| Receipt Date | 5-3-12 |
| Temperature (°C) | 2.0 |
| pH | 8.0 |
| Dissolved oxygen (mg/L) | 10.4 |
| Salinity (‰) | 29.0 |

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, OR

Date of Collection: 5-2-12

Water Quality: Salinity: 34.0 ‰, pH: 8.0

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, aerated, salinity adjusted with 100 ppt brine prepared on 3-19-12.

BRINE USED FOR SALINITY CONTROL

None used.

TEST ORGANISMS

Species: Sea urchin (*Strongylocentrotus purpuratus*).

Age: Sperm were used immediately after seawater activation.

Source: Marinus Scientific, Newport Beach, CA.

Acclimation: Adults were received on 2-1-12 and held in flowing seawater until used for testing. Holding conditions prior to testing averaged: temperature, $11.9 \pm 0.9^\circ\text{C}$; pH, 8.0 ± 0.1 ; salinity, 26.2 ± 2.7 ppt; and dissolved oxygen, 8.2 ± 0.7 mg/L.

Source of Gametes: 1 female, 2 males

TEST PROCEDURES AND CONDITIONS

Test Chambers: 16 mm x 100 mm unwashed new borosilicate disposable glass test tubes containing 5 ml of test solution.

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01 and 0% (control).

Brine Control: None used

Replicates/Treatment: 4

Eggs per Test Container: 1000

Sperm:Egg Ratio: 100:1

Sperm Exposure Time: 20 minutes

Time for Fertilization: 20 minutes

Volume of Subsamples Taken for Counting: 1 ml

Water Volume Changes: None (non-renewal static test).

Aeration: None

Feeding: None

Effects Criteria: The effect criterion was absence of fertilization as indicated by lack of a fertilization membrane in the preserved eggs.

Water Quality and Other Test Conditions: Temperature, 11.8°C ; pH, 8.0 ± 0.0 ; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 8.4 ± 0.1 mg/L. Photoperiod: NA

DATA ANALYSIS METHODS

The proportion of fertilized eggs was calculated for each treatment replicate from the raw data and the means were obtained for each treatment level. The latter were then corrected for control response using Abbott's formula. The EC50 was calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber methods. An IC25 was calculated by linear interpolation with bootstrapping. NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v.1.7.0revW, Tidepool Scientific Software. Toxic units (TU_c) were computed as $100/\text{NOEC}$, $100/\text{EC50}$, or $100/\text{IC25}$.

PROTOCOL DEVIATIONS

None.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using sodium azide to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3025

Reference Toxicant and Source: Sodium azide (Sigma Lot No. 68F-0834), 1.0 mg/mL stock prepared on 5-3-12.

Test Date: 5-3-12

Dilution Water Used: Yaquina Bay, OR, Salinity 34.0 ‰, pH 8.0.

Results: EC50, 191 mg/L; NOEC, 47 mg/L; and IC25, 148 mg/L. The EC50 result is slightly above the laboratory's control chart warning limits (EC50, 83.2 – 189 mg/L), but is within the control chart upper action limit (216 mg/L). Control limits of ± 2 SD will be exceeded 5% of the time by chance alone, and there was no evidence that these organisms were unusual in any way.

TEST RESULTS

A detailed tabulation of the test results is given in Table 1. The biological effects, given as the NOEC, LOEC, and EC50 and IC25 for inhibition of fertilization are shown below.

| | |
|-----------------------|------------------------|
| NOEC (%) | 0.16 ($TU_c = 625$) |
| LOEC (%) | >0.16 ($TU_c < 625$) |
| EC50 (%) | >0.16 ($TU_c < 625$) |
| (95% C.I.) | --- |
| Method of Calculation | By Data Inspection |
| IC25 (%) | >0.16 ($TU_c < 625$) |
| (95% C.I.) | --- |
| Method of Calculation | Linear Interpolation |

DISCUSSION/CONCLUSIONS

The NOEC in this study was 0.16 % effluent, and the EC50 and IC25 for fertilization were both >0.16 %.

The reference toxicant test results exceeded the control chart limits for the EC50. The EC50 was 191 mg/L, which is slightly above the upper warning limit of 189 mg/L, but within the upper action limit of 216 mg/L. No other problems could be found with the test organisms or the testing procedure.

Table 2 shows the results of the egg-effluent control and egg-control tests. No fertilization response was observed in the egg only, no-sperm control or in the egg-effluent, no-sperm control.

STUDY APPROVAL

Gay Buhl 6/19/12
Project Manager/Study Director Date

Julie R. Jone 6-19-12
Quality Assurance Unit Date

Shirley K. Nemeth 6/15/12
Assistant Laboratory Director Date

Table 1. Fertilization response of Sea urchin, *Strongylocentrotus purpuratus*, sperm exposed to XTO Energy Platform A non-contact cooling water sample.

| Effluent Conc. (%) | Replicate | Eggs Counted | | Proportion Fertilized | |
|--------------------|-----------|--------------|--------------|-----------------------|--------|
| | | Fertilized | Unfertilized | | Mean** |
| 0.16 | 1 | 98 | 2 | 0.980 | 0.990 |
| | 2 | 99 | 1 | 0.990 | |
| | 3 | 99 | 1 | 0.990 | |
| | 4 | 100 | 0 | 1.000 | |
| 0.08 | 1 | 99 | 1 | 0.990 | 0.983 |
| | 2 | 94 | 6 | 0.940 | |
| | 3 | 100 | 0 | 1.000 | |
| | 4 | 100 | 0 | 1.000 | |
| 0.04 | 1 | 100 | 0 | 1.000 | 0.995 |
| | 2 | 99 | 1 | 0.990 | |
| | 3 | 99 | 1 | 0.990 | |
| | 4 | 100 | 0 | 1.000 | |
| 0.02 | 1 | 97 | 3 | 0.970 | 0.978 |
| | 2 | 98 | 2 | 0.980 | |
| | 3 | 99 | 1 | 0.990 | |
| | 4 | 97 | 3 | 0.970 | |
| 0.01 | 1 | 95 | 5 | 0.950 | 0.985 |
| | 2 | 99 | 1 | 0.990 | |
| | 3 | 100 | 0 | 1.000 | |
| | 4 | 100 | 0 | 1.000 | |
| Control | 1 | 98 | 2 | 0.980 | 0.978 |
| | 2 | 98 | 2 | 0.980 | |
| | 3 | 96 | 4 | 0.960 | |
| | 4 | 99 | 1 | 0.990 | |

** Treatment mean significantly ($P < 0.05$) different from the control mean

Table 2. Response of egg-effluent controls (no sperm) and egg-controls (no sperm, dilution water only).

| Description | Replicate | Eggs Counted | | Proportion Fertilized | |
|----------------------|-----------|--------------|--------------|-----------------------|-------|
| | | Fertilized | Unfertilized | | Mean |
| Egg-effluent control | 1 | 0 | 100 | 0.000 | 0.000 |
| | 2 | 0 | 100 | 0.000 | |
| | 3 | 0 | 100 | 0.000 | |
| | 4 | 0 | 100 | 0.000 | |
| Egg control | 1 | 0 | 100 | 0.000 | 0.000 |
| | 2 | 0 | 100 | 0.000 | |
| | 3 | 0 | 100 | 0.000 | |
| | 4 | 0 | 100 | 0.000 | |

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-145

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform A – non-contact cooling water.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Aq. Toxicologist; M.S. Redmond, M.S., Aq. Toxicol.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 5-5-12, 1320 hrs.

Test Ending: 5-7-12, 1430 hrs.

Disposition of Study Records: All raw data, reports and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy – Platform A – non-contact cooling water. Details are as follows:

| | |
|-------------------------|--------|
| NAS Sample No. | 4079G |
| Collection Date | 5-4-12 |
| Receipt Date | 5-5-12 |
| Temperature (°C) | 1.2 |
| pH | 8.0 |
| Dissolved oxygen (mg/L) | 9.8 |
| Salinity (‰) | 29.0 |

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 5-4-12

Water Quality: Salinity, 30.5 ‰; pH, 8.1

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, aerated, and salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 1.7 hours post-fertilization.

Source: Carlsbad Aquafarm, Carlsbad, CA.

Conditioning: Adult mussels were received on 5-5-12 and used directly from shipping container.

Source of Gametes: 4 females and 2 males.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.

Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).

Brine Control: None used

Replicates/Treatment: 4

Initial Concentration of Test Organisms: 20.3/ml.

Volume of Subsamples Taken for Counting: NA

Water Volume Changes per 24 hr: None (non-renewal static test).

Aeration: None

Feeding: None

Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.

Water Quality and Other Test Conditions: Temperature, $15.6 \pm 0.1^\circ\text{C}$; pH, 8.2 ± 0.0 ; salinity, $30.0 \pm 0.0\text{‰}$; and dissolved oxygen, $8.1 \pm 0.1\text{ mg/L}$. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Kärber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0revW, Tidepool Scientific Software. Toxic units (TU_x) were computed as $100/\text{NOEC}$, $100/\text{EC50}$, or $100/\text{IC25}$.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3039

Reference Toxicant and Source: Copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Argent Lot No. 0195. Concentrated stock prepared 6-28-10.

Test Date: 5-5-12

Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.5 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.41 µg/L. The EC50 results are within the laboratory's control chart warning limits (9.17 – 12.4 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

| | Combined Proportion Normal | Survival |
|-----------------------|-------------------------------|----------------------|
| NOEC (%) | 0.16 ($TU_c=625$) | 0.16 ($TU_c=625$) |
| LOEC (%) | >0.16 ($TU_c<625$) | >0.16 ($TU_c<625$) |
| EC50/LC50 (%) | >0.16 ($TU_c<625$) | >0.16 ($TU_c<625$) |
| (95% C.I.) | --- | --- |
| Method of Calculation | By Data Inspection | By Data Inspection |
| IC25 (%) | >0.16 ($TU_c<625$) | |
| (95% C.I.) | --- | |
| Method of Calculation | Linear Interpolation | |

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

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Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – Platform A – non-contact cooling water.

| Test Material | | | | | Combined Proportion Normal* | | Proportion Survived* | |
|-------------------|-------|-------|------|-------|-----------------------------------|-------|-------------------------|-------|
| Concentration (%) | Repl. | Norm. | Abn. | Total | Mean | | Mean | |
| 0.16 | 1 | 180 | 9 | 189 | 0.887 | | 0.931 | |
| | 2 | 199 | 5 | 204 | 0.980 | | 1.000 | |
| | 3 | 195 | 5 | 200 | 0.961 | | 0.985 | |
| | 4 | 173 | 3 | 176 | 0.852 | 0.920 | 0.867 | 0.946 |
| 0.08 | 1 | 194 | 5 | 199 | 0.956 | | 0.980 | |
| | 2 | 185 | 6 | 191 | 0.911 | | 0.941 | |
| | 3 | 211 | 10 | 221 | 0.955 | | 1.000 | |
| | 4 | 186 | 7 | 193 | 0.916 | 0.935 | 0.951 | 0.968 |
| 0.04 | 1 | 205 | 11 | 216 | 0.949 | | 1.000 | |
| | 2 | 209 | 13 | 222 | 0.941 | | 1.000 | |
| | 3 | 202 | 4 | 206 | 0.995 | | 1.000 | |
| | 4 | 200 | 3 | 203 | 0.985 | 0.968 | 1.000 | 1.000 |
| 0.02 | 1 | 205 | 4 | 209 | 0.981 | | 1.000 | |
| | 2 | 189 | 3 | 192 | 0.931 | | 0.946 | |
| | 3 | 205 | 5 | 210 | 0.976 | | 1.000 | |
| | 4 | 187 | 10 | 197 | 0.921 | 0.952 | 0.970 | 0.979 |
| 0.01 | 1 | 158 | 10 | 168 | 0.778 | | 0.828 | |
| | 2 | 209 | 12 | 221 | 0.946 | | 1.000 | |
| | 3 | 209 | 5 | 214 | 0.977 | | 1.000 | |
| | 4 | 184 | 11 | 195 | 0.906 | 0.902 | 0.961 | 0.947 |
| Normal Control | 1 | 204 | 5 | 209 | 0.976 | | 1.000 | |
| | 2 | 190 | 10 | 200 | 0.936 | | 0.985 | |
| | 3 | 192 | 6 | 198 | 0.946 | | 0.975 | |
| | 4 | 199 | 7 | 206 | 0.980 | 0.960 | 1.000 | 0.990 |

* Based on an average initial count of 203 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal > average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different ($P \leq 0.05$) from the control.